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Adrenergic modulation of the type 1 IP_3 receptors in the rat heart

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Abstract

Inositol 1,4,5-trisphosphate (IP_3) receptors are calcium-releasing channels localized on the sarcoplasmic reticulum. IP_3 receptors mediate the calcium mobilizing effect of a wide range of hormones, cytokines, and neurotransmitters and play an important role in variety of cell functions. The aim of this work was to study, how partial depletion of catecholamines affects the gene expression and protein levels of the type 1 IP_3 receptors in rat heart. The type 1 IP_3 receptor mRNA levels were studied in the left cardiac atrium and ventricle of rats treated with 6-hydroxydopamine (6-OHDA) in control and stressed conditions. The 6-OHDA produces anatomical and functional denervation resulting in decreased levels of noradrenaline and adrenaline. We also used corticoliberin (CRH) knockout mice, where secretion of adrenaline is significantly suppressed. Administration of 6-OHDA significantly decreases mRNA levels of the type 1 IP_3 receptor in both, the left atrium and the left ventricle, while the gene expression of the sarcoplasmic reticular Ca^{2+}-ATPase (SERCA 2) was unaffected. CRH knockout mice possess markedly lower levels of the type 1 IP_3 receptor mRNA compared to wild-type mice in both, control and stressed conditions. These data point to the adrenergic modulation of the type 1 IP_3 receptors in the rat hearts.

Keywords: Type 1 IP_3 receptor; 6-hydroxydopamine; Cardiac atria; Cardiac ventricle; CRH knockout mouse; Catecholamine

1. Introduction

The inositol 1,4,5-trisphosphate receptors (IP_3 receptors) are intracellular membrane channels essential for calcium release from intracellular stores. Physiological function of IP_3 receptors in the heart is not completely understood yet. Compared to ryanodine receptors, responses to IP_3 are slow and weak, and the calcium so generated does not contribute to calcium-induced calcium release. Thus, IP_3 is less likely to be important in regulating beat-to-beat changes in intracellular calcium under physiological conditions. All three types of IP_3 receptors (types 1, 2 and 3) are expressed in the heart. Type 2 IP_3 receptor is most abundant in cardiomyocytes, while type 1 occurs predominantly in cardiac ganglia [1]. The neurons of these ganglia, situated at the outer surface of the atria, receive input from efferent vagal fibres and they innervate the atrial musculature, in particular the sinoatrial and atrioventricular nodes, thus playing a crucial role in the regulation of heart rate [2,3]. Type 1 IP_3 receptors might be involved in the activation of neurons in cardiac parasympathetic ganglia by sympathetic stimulation and therefore adrenergic modulation of these receptors might be important in this process. Modulation of IP_3 receptors during some cardiac diseases was also proposed. IP_3 receptors mediate the calcium mobilizing effect of a wide range of hormones, cytokines and neurotransmitters (for review see [4]). Among them, catecholamines acting through the α- and β-adrenergic receptors play an unmistakable role in the cardiac function. Stimulation of the α1 adrenergic receptors (α1AR; which are abundant in myocardium) results in the activation of phospholipase C and production of the IP_3. In addition, α1AR activation induces transcriptional activation of early- and late-response genes [5]. In several species α1AR density is higher in the ventricular than in atrial tissues [6]. The heart expresses also β1 and β2AR; both subtypes increase cardiac frequency and contractility [7]. The βAR system activates adenylyl cyclases and thereby elevates cAMP.

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Primary target for the cAMP is protein kinase A (PKA), which stimulates also phospholamban [7]. Phospholamban is an inhibitor of sarcoplasmic reticular Ca^{2+}-ATPase (SERCA) and both these proteins can affect IP_3 receptors through the loading of calcium stores. Therefore, catecholamines can affect the IP_3 receptors by several mechanisms.

6-hydroxydopamine (6-OHDA) is neurotoxin that destroys catecholaminergic terminals [8]. Administration of 6-OHDA to rats produces anatomical and functional noradrenergic denervation and induces compensatory hyperreactivity of the suprarenal chromaffin cells. These effects are manifested by the increase in the activity of catecholamine synthesizing enzymes [9]. In the rat heart, a significant increase in the phenylethanolamine-N-methyltransferase (PNMT) activity was found in 6-OHDA-treated rats, compared to controls [10].

Corticotropin-releasing hormone (corticoliberin; CRH) is thought to be important for the function of the adrenomedullary catecholaminergic system. Thus, CRH knockout mice represent a good experimental model to study regulatory pathways and physiological importance of the altered hypothalamic–pituitary–adrenocortical (HPA) axis, and also stress response in mammals (for review see [11]). CRH deficiency leads to impaired gene expression and catecholaminergic enzyme activity levels, and to weakened adrenaline synthesis and secretion in the adrenal medulla, possibly due to impaired adrenocortical corticosterone production [12]. CRH knockout (CRH KO) mice exhibit also blunted glucocorticoid assembly. It is therefore possible that the adrenal medulla could contribute to the elevation of plasma noradrenaline [12].

Stress is one of major contributors to the development of cardiovascular diseases and psychiatric illnesses. Therefore, it is crucial to clarify mechanisms involved in the conversion of brief beneficial responses to stressors into prolonged detrimental consequences. We have already shown different effect of single and repeated immobilization stress on gene expression of the IP_3 receptors. After the single immobilization stress, the gene expression of the type 1 IP_3 receptors was increased [13], while after the repeated immobilization stress for seven times, both gene expression and protein of the type 1 IP_3 receptors were decreased significantly in the left atrium of rats [14].

In this work we focused on the effect of chemical sympathectomy by 6-OHDA on the type 1 IP_3 receptors. The aim of this work was to study, how partial depletion of catecholamines can affect the gene expression and protein levels of the type 1 IP_3 receptors in the rat heart.

2. Material and methods

2.1. Animals

The Ethic Committee of the Institute of Experimental Endocrinology, Slovak Academy of Sciences approved all presented experiments.

Male Sprague–Dawley rats (cca 350 g, Charles River, Suzfeld, Germany) 4 months old were used for 6-OHDA experiment. Prior to experiments, animals were housed for 1 week, four animals per cage in a controlled environment (22±2 °C, 12 h light/dark cycle, light on at 6.00 a.m.). Food and water were available ad libitum. One experimental group of rats was treated two times (second administration after 24 h) by 6-OHDA, each dose was 100 mg per 1 kg of body weight. Sham vehicle-treated and 6-OHDA-treated animals were divided into 2 groups and one control group and one 6-OHDA group of rats was immobilized once for 2 h as described previously [15] and decapitated 3 h after the IMO termination. Each group was composed of 10 animals. Animals were sacrificed by decapitation 2 weeks after the last 6-OHDA administration; left ventricle and atrium was withdrawn and immediately frozen in the liquid nitrogen.

CRH KO mouse line was originally obtained from the Harvard Medical School, Department of Endocrinology, Boston, USA [16]. Presence of CRH KO allele was specified by using tail DNA isolation with subsequent PCR. Male CRH knockout mice approximately 12 weeks old were used in this experimental study. The presence of CRH KO allele was specified using tail DNA isolation with subsequent PCR [14,17]. Mice were maintained under controlled environmental conditions (22±1 °C, 12-h light/dark cycle, lights on at 6:00 A.M.). Immobilization stress was performed as described previously [15], however, the size of immobilization boards was modified for mice. Repeated stress was achieved by immobilizing the animals for 7 days, 2 h daily with subsequent decapitation 3 h after the last, seventh immobilization (7×). Control mice were sacrificed immediately after removal from their home cages.

2.2. RNA isolation and relative quantification of mRNA levels by reverse transcription and subsequent polymerase chain reaction (RT-PCR)

Population of total RNAs was isolated by TRI Reagent (MRC Ltd.). Briefly, tissue samples were homogenized by tissue homogenizer (Biospec Products Inc.) in TRI Reagent and after 5 min the homogenate was extracted by chloroform. RNAs in the aqueous phase were precipitated by isopropanol. RNA pellet was washed with 75% ethanol and stored under 96% ethanol at −70 °C. The purity and integrity of isolated RNAs was checked on GeneQuant Pro spectrophotometer (Amersham Biosciences). Reverse transcription was performed using 1.5 μg of total RNAs and Ready-To-Go You-Prime First-Strand Beads (Amersham Biosciences) with pd(N_6) primer. PCR specific for the type 1 IP_3 receptor (IP_3R1) was carried out afterwards using primers (Table 1; GI: 1055286) described in Genazzani et al. [18]. Sarco(endo)plasmic reticulum Ca^{2+}-ATPase (SERCA2; GI: 8392934) gene and Phospholamban (PLB; GI: 313809) gene were amplified with primer sets according to Seth et al. [19]. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH; [20]) expression was used as a housekeeper gene control for semi-quantitative evaluation of PCR. All used primer sequences, together with corresponding position and fragment size for each amplified gene, are listed in Table 1. Each PCR program

Table 1
Sequences of primers, their position and product size

Primer	Primer sequences	Position	Product size (bp)
IP_3R1A	5′-GTG GAG GTT TCA TCT GCA AGC-3′	70–90	
IP_3R1B	5′-GCT TTC GTG GAA TAC TCG GTC-3′	456–476	410
SERCA2A	5′-ATT GTT CGA AGT CTG CCT TCT GTG G-3′	1489–1522	
SERCA2B	5′-CAT AGG TTG ATC CAG TTA TGG TAA A-3′	1648–1672	174
PLB-A	5′-GAA CCT CCA GAA CCT CTT TAT CAA T-3′	251–276	
PLB-B	5′-TGA CCC TTC ACG ACG ATG TCC CAG C-3′	426–450	199
GAPDH1	5′-AGA TCC ACA ACG GAT ACA TT-3′	795–814	
GAPDH2	5′-TCC CTC AAG ATT GTC AGC AA-3′	506–525	309

(IP_3R1) type 1 IP_3 receptor, (SERCA2) sarco(endo)plasmic reticulum Ca^{2+}-ATPase, (PLB) phospholamban, (GAPDH) glyceraldehyde 3-phosphate dehydrogenase.

started with initial denaturation at 94 °C for 5 min, followed by 25 (for SERCA 2 and PLB), 30 (for GAPDH) or 35 (for IP_3R1) cycles of denaturation at 94 °C for 1 min, annealing at 60 °C for 1 min, and polymerization at 72 °C for 1 min. PCRs were terminated by final polymerization at 72 °C for 7 min. All PCR products were analyzed on 2% agarose gels. Optical density (od/mm^2) of individual bands was measured by Kodak camera and Biometra software.

2.3. Western blot analysis

The IP_3R1 protein was determined in crude membrane fraction from the left ventricle by Western blot analysis. Protein concentration was determined according to Lowry et al. [21]. 100 μg of protein extract from cardiac atria and ventricles were used for Western blot analysis, as described in Zacikova et al. [22]. Using a polyclonal antiserum specific for IP_3R1 receptor we detected immunoreactive protein of approximately 240 kDa. Specific anti-IP_3-receptor polyclonal antibody (rabbit, dilution 1:1000 in 5% non-fat dry milk; Abcam) was raised against a synthetic peptide corresponding to the C-terminus of human IP_3 receptor (amino acids 1829–1848). This sequence is 100% conserved in human, mouse, and rat type 1 IP_3 receptor protein. Antibody cross-reacts with dog and rat and is expected to cross-react with human and mouse due to sequence homology (100%). After the incubation with secondary anti-rabbit antibody conjugated to the horseradish peroxidase (1:5000), immunoreactive proteins were visualized by ECL detection system (Amersham Biosciences). As a control of equal loading, levels of GAPDH were determined with mouse anti GAPDH monoclonal antibody (dilution 1:500; Chemicon).

2.4. Statistical analysis

Each value represents the average for 5-10 animals. Results are presented as means ± S.E.M. Statistical differences among groups were determined by one-way analysis of variance (ANOVA). Statistical significance $P<0.05$ was considered to be significant. For multiple comparisons, an adjusted t test with P values corrected by the Bonferroni method was used (Instat, GraphPad Ssoftware, USA).

3. Results

Treatment with the 6-OHDA decreased significantly levels of the heart noradrenaline in both, control (from 123 ± 32 to 44 ± 12 μg/g tissue) and immobilized (from 122 ± 25 to 43 ± 6 μg/g tissue) group (Fig. 1A). Tissue adrenaline was also decreased by 6-OHDA treatment (from 7.0 ± 3.1 to 2.8 ± 1.0 μg/g tissue; Fig. 1B). In the group of sham-treated rats, concentration of tissue adrenaline was significantly elevated by single immobilization (from 7.0 ± 3.1 to 19.7 ± 3.6 μg/g tissue), but immobilization-induced increase in adrenaline levels in 6-OHDA-treated rats was highly suppressed (from 19.7 ± 3.6 to 5.9 ± 1.0 μg/g tissue). The type 1 IP_3 receptor mRNA levels in left atria and left ventriculi of rat myocardium revealed significant differences between control animals and animals treated with the 6-OHDA (Fig. 2). In left atria administration of the 6-OHDA significantly decreased IP_3 receptors of type 1 mRNA levels in both, control (from 44.8 ± 2.3 to 26.6 ± 2.3 a.u.) and one time immobilized group of rats (from 47.7 ± 3.1 to 30.2 ± 1.8 a.u.; Fig. 2A). In the left ventriculi, similar decrease in IP_3R1 mRNA (Fig. 2B) and protein (Fig. 2C) levels was observed. Control IP_3R1 mRNA levels decreased (from 77.8 ± 2.1 to 41.3 ± 5.3 a.u.) and IP_3R1 gene expression of immobilized animals was lowered (from 103.9 ± 11 to 73.9 ± 9.8 a.u.) in 6-OHDA-treated group of rats, compared to sham-treated rats. The type 1 IP_3R1 protein was also lower after 6-OHDA treatment in both, control (from

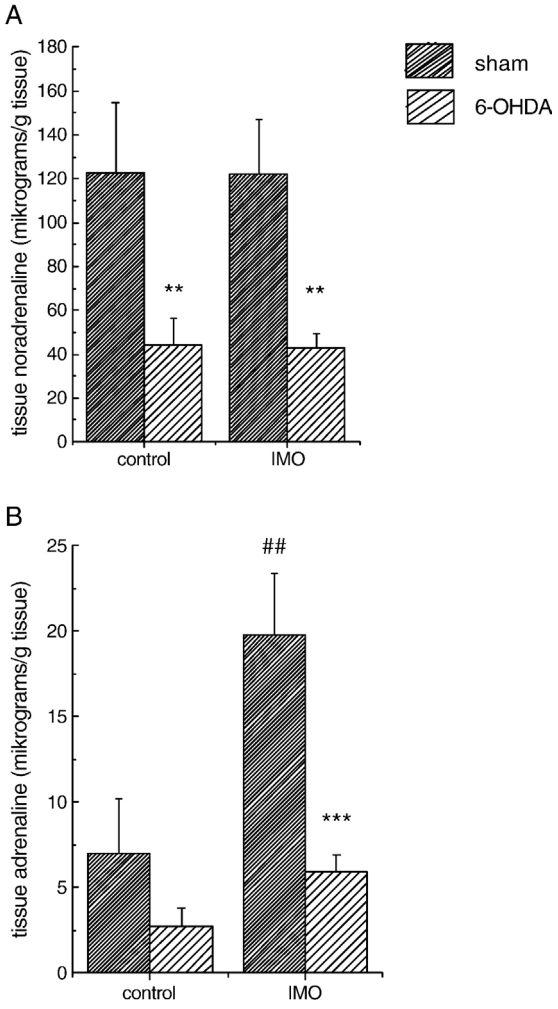

Fig. 1. Noradrenaline (A) and adrenaline (B) levels in the left ventricle of sham- (fine striped columns) and 6-OHDA- (coarse striped columns) treated rats. Both tissue noradrenaline and adrenaline were depressed in 6-OHDA-treated animals in control conditions and also after the single exposure to immobilization stress (IMO). Results are presented as a mean ± S.E.M. Statistical differences among groups were determined by one-way analysis of variance (ANOVA). Statistical significance between sham-treated and 6-OHDA-treated rats was considered as $^{**}P<0.01$ and $^{***}P<0.001$. Statistical significance between control and immobilized group of rats was defined as $^{\#\#}P<0.01$.

786 ± 39 to 369 ± 168 a.u.) and immobilized (from 1108 ± 144 to 686 ± 62 a.u.) group. Nevertheless, 6-OHDA treatment did not alter levels of the SERCA and phospholamban (Table 2) in the left atria/ventriculi, neither in control conditions, nor after the stress exposure.

CRH knockout mice manifested slightly increased levels of plasma noradrenaline compared to wild-type mice (from 6189 ± 608 to 8396 ± 1049 pg/ml; Fig. 3A), but rapid decrease in plasma adrenaline (from 6318 ± 1131 to 2277 ± 231 pg/ml; Fig. 3B) has been observed. In the left atrium of CRH KO mice, the gene expression of type 1 IP_3 receptor was significantly lower than in left atrium of wild-type animals (19.2 ± 3.0 vs. 6.1 ± 1.1 a.u.; Fig. 4A). After the repeated immobilization for 7-times levels of the IP_3R1 mRNA significantly decreased in wild-type group of mice compared to control (from 19.2 ± 3.0 to 6.9 ± 1.1 a.u.), while in CRH KO

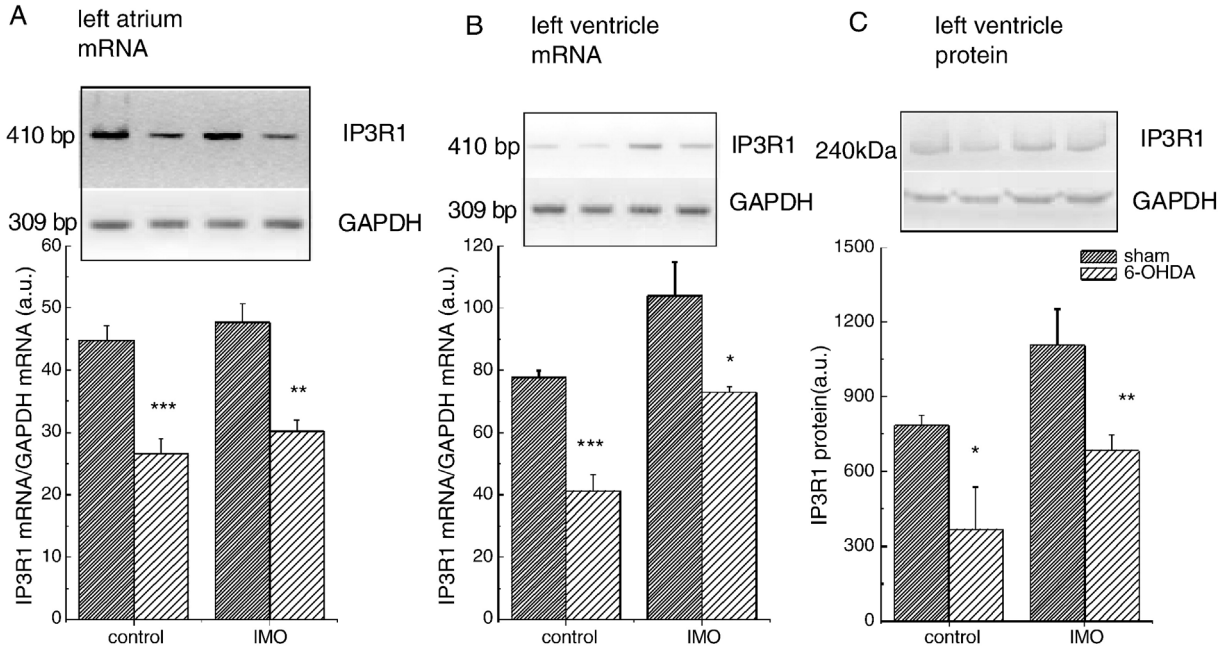

Fig. 2. The IP_3R1 mRNA levels in the left atrium (A), the left ventricle (B) and left ventricular protein levels (C) of sham- (fine striped columns) and 6-OHDA- (coarse striped columns) treated rats. In the upper part, representative results of IP_3R1 and GAPDH from gels are shown. The mRNA levels of the IP_3R1 were decreased in both, left atrium and left ventricle of control and also single immobilized (IMO) rats. Also IP_3R1 protein was decreased after 6-OHDA treatment. Results are presented as mean ± S.E.M. Statistical differences among groups were determined by one-way analysis of variance (ANOVA). Statistical significance between sham-treated and 6-OHDA-treated rats was determined as $*P<0.05$, $**P<0.01$ and $***P<0.001$.

mice repeated immobilization did not affect the gene expression of the IP_3R1 at all (from 6.1 ± 1.1 to 6.4 ± 1.4 a.u.). Similarly, in the left ventricles CRH knockouts expressed IP_3R1 mRNA in significantly lower levels than their wild-type counterparts (Fig. 4B; wt: 19.2 ± 0.9 a.u. vs. CRH KO: 8.1 ± 1.0 a.u.). Repeated immobilization for 7-times significantly decreased IP_3R1 gene expression in wild-type animals compared to their controls (from 19.2 ± 0.9 a.u. to 6.4 ± 0.6 a.u.). Decrease of the gene expression of the type 1 IP_3 receptor due to 7- times repeated immobilization was observed also in the CRH KO group of mice (from 8.1 ± 1.0 a.u. to 4.6 ± 0.8 a.u.).

4. Discussion

In this paper, we demonstrate that partial depletion of catecholamines decreased significantly mRNA and protein levels of the type 1 IP_3 receptors in the heart in both, control conditions and after the exposure to immobilization stress for two h. Interestingly, mRNA levels of phospholamban and SERCA 2, responsible for calcium loading of the intracellular stores were unaffected by catecholamine depletion.

In cardiac muscle, several plasma membrane receptors (α1AR, muscarinic, angiotensin II, endothelin, etc.) are coupled to phosphatidylinositol 4,5-bisphosphate (PIP2) turnover [23–26]. PIP2 is cleaved by phospholipase C and produces IP_3 and diacylglycerol. The IP_3 activates IP_3 receptors. The mRNA encoding the intracellular IP_3 receptor has been detected in the heart [13,27,28]. However, the physiological role of calcium release through the IP_3 receptors in the heart remains elusive. Nevertheless, in Purkinje myocytes, IP_3-induced calcium release appears to be causally related to both increase in automaticity and generation of some arrhythmias after α1-adrenergic stimulation [26].

Catecholamines are distinguished modulators of the cardiac function, because of their strong inotropic and chronotropic effect. Rapacciuolo and coworkers [29] clearly established the important role of noradrenaline and adrenaline in the induction of cardiac hypertrophy and suggested that blocking the action of adrenaline and noradrenaline would not only be favorable with regard to cell survival, but it would also inhibit cardiac hypertrophy. 6-OHDA is a potent neurotoxin used extensively to produce chemical sympathectomy in experimental animals and an important tool for the understanding of the adrenergic mechanism [9,30,31]. In accordance with other laboratories [9,10,32], we observed depletion of noradrenaline levels in 6-OHDA-treated rats compared to controls. Adrenaline levels were also decreased, since noradrenaline is a substrate for adrenaline production. 6-OHDA treatment of rats results in the suppression of the gene expression and protein levels of the type 1 IP_3 receptors both in the left atrium and left ventricle. Mechanism, by which chemical sympathectomy by 6-OHDA reduces the level of IP_3R1, is not clear. Several suggestions about the possible mechanism of this effect should be taken in the consideration. Firstly, since the type 1 IP_3 receptor is

Table 2
SERCA 2 and phospholamban mRNA levels in the left atrium (LA) and the left ventricle (LV)

mRNA (a.u.)	Control	6-OHDA	Control IMO	6-OHDA IMO
SERCA 2 LA	887 ± 227	884 ± 100	900 ± 230	888 ± 85
SERCA2 LV	538 ± 71	544 ± 104	512 ± 23	585 ± 64
Phospholamban LV	136 ± 11	139 ± 9	145 ± 5	140 ± 4

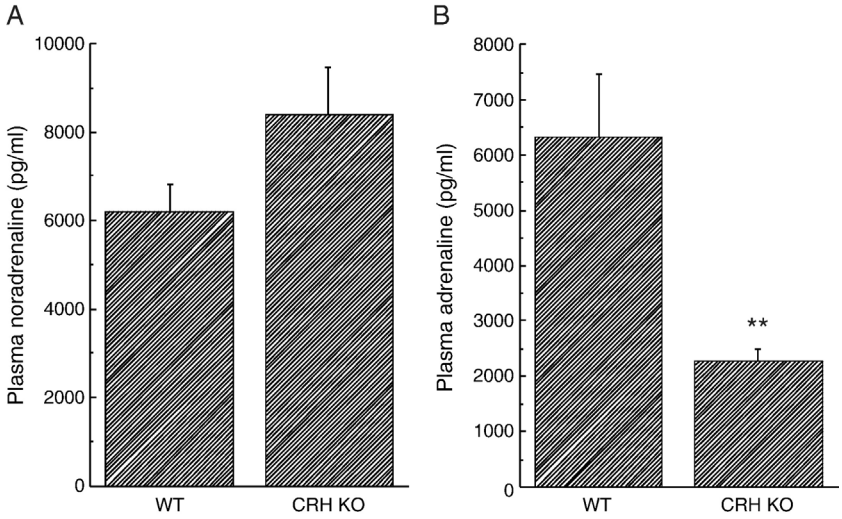

Fig. 3. Plasma noradrenaline (A) and adrenaline (B) levels in wild-type and CRH KO mice. Plasma noradrenaline was not significantly changed, although the tendency of increase was observed in CRH KO mice compared to wild-type controls. Plasma adrenaline was significantly depressed in CRH KO animals. Results are presented as a mean±S.E.M. Statistical differences among groups were determined by one-way analysis of variance (ANOVA). Statistical significance was defined as $^{**}P<0.01$.

localized predominantly in cardiac ganglia [1] chemical sympathectomy can affect IP_3 receptors in neuronal cells as well, which might result in downregulation of their gene expression. Also, it was previously reported that different gene expression profiles in cardiomyocytes were observed after noradrenaline infusion [32,33]. Golden et al. [34] reported that noradrenaline treatment alters DHPR mRNA and protein levels, possibly through the AP1 transcription factor. Since type 1 IP_3 receptor promoter has two AP1 transcription factors [35], we might speculate that chemical sympathectomy, which results in depletion of noradrenaline levels affects these receptors also through the AP1 transcription factors. Nevertheless, this proposal remains to be experimentally verified.

We also tested the hypothesis, whether mRNA levels of SERCA2 are also modified. We have found that gene expression of the SERCA2 and phospholamban was not altered due to 6-OHDA treatment. Ishibashi and coworkers [36] showed that noradrenaline increased intracellular calcium even in the absence of extracellular calcium. Depletion of calcium stores has been reported to activate the cation channels [37,38]. Thus, activation of cation channels of rat cardiac ganglion neurons depends on calcium released from calcium stores via IP_3 receptors, but not on the depletion of the calcium store per se [36]. Depleted noradrenaline would probably decrease demands for intracellular calcium released through the type 1 IP_3 receptors, which in turn results in the decreased gene expression of these intracellular calcium channels.

Cardiac β-adrenergic receptors, which respond to neuronally released and circulating catecholamines, are important regulators of cardiac function. β-AR mediated stimulation of adenylyl cyclase increases cellular levels of cAMP and, in turn, the phosphorylation via cAMP dependent protein kinase of proteins such as phospholamban, calcium channels, and contractile proteins. Phosphorylation of these proteins alters their activity and leads to a functional response [39]. The phosphorylation of IP_3 receptors mediated by cAMP dependent

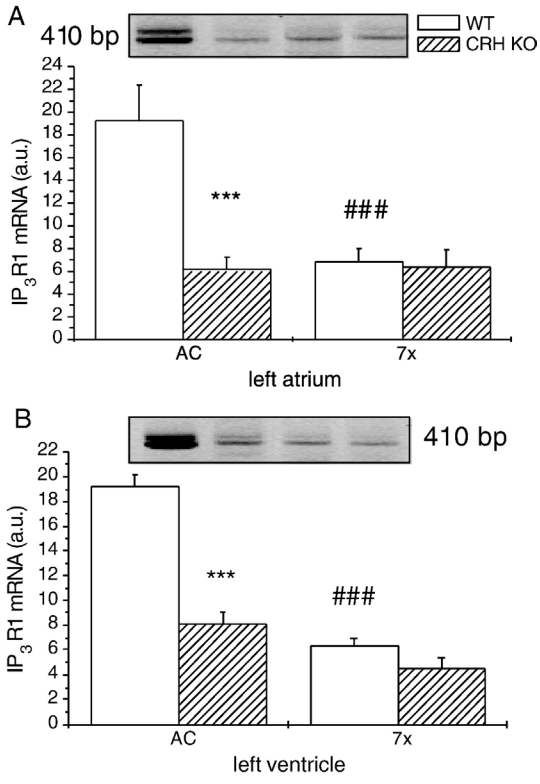

Fig. 4. The type 1 IP_3 receptor mRNA levels in the left atrium (A) and the left ventricle (B) of wild-type (empty columns) and CRH KO (striped columns) mice. The mRNA levels of the IP_3R1 were decreased significantly in both, left atrium and left ventricle of CRH KO mice compared to wild-type mice. Repeated immobilization stress decreased significantly mRNA levels of the IP_3R1 in both, left atrium and left ventricle (empty columns). After the repeated immobilization for seven times, no significant difference was observed among wild-type and CRH KO mice in both, left atrium and the left ventricle. Results are presented as mean±S.E.M. Statistical differences among groups were determined by one-way analysis of variance (ANOVA). Statistical significance between wild-type (WT) control and CRH KO control group was defined as $^{***}P<0.001$ and between WT control and IMO control was defined as $^{###}P<0.001$.

protein kinase A has been demonstrated in numerous cells and has been proposed as an explanation for the diverse abilities of distinct G-protein-coupled receptors that signal through the IP_3 receptors to initiate and/or sustain intracellular calcium oscillations [40]. Impaired activation of βAR signaling results into a reduction of the amount of cAMP, which in turns binds to the regulatory subunits of PKA and phosphorylation of a number of protein targets can be rendered. Thus, decreased levels of adrenaline in our experiments can reduce gene expression of the IP_3 receptors also via βAR.

Prolonged stress is one of the major contributors to the development of several diseases, such as psychiatric illnesses or those of cardiovascular origin. Immobilization stress is one of the most potent stressors, since it activates both pathways of the sympathoadrenal system. Single exposure to the immobilization stress affects the gene expression and thus the protein levels of IP_3 receptors differently in various tissues [13,41,42]. Repeated immobilization decreased a gene expression of the type 1 and 2 IP_3 receptors and also protein levels of the IP_3 receptors in the rat heart [14]. In the hearts of wild-type mice we observed the same decrease in gene expression of the type 1 IP_3 receptors as we published in Krepsova et al. [14] in rats. Modulatory effect of immobilization stress on the type 1 IP_3 receptors might be part of the compensatory mechanism, by which heart respond to the development of the pathophysiological state. Nevertheless, this proposal remains to be verified.

Many stressors increase the secretion of catecholamines from the adrenal medulla and sympathetic nerve terminals. Such stressors also potentiate the secretion of the hypothalamic–pituitary–adrenocortical (HPA) axis hormones, glucocorticoids from the adrenal cortex via stimulation by the hypothalamic neuropeptide, CRH [43]. CRH contributes to the regulation of the adrenomedullary catecholaminergic system under stressful conditions through both the HPA axis and sympathetic nervous system. CRH deficiency leads to impaired PNMT gene expression and enzyme activity levels, and to impaired adrenaline synthesis and secretion in the adrenal medulla [12]. We also observed significantly depressed levels of plasma adrenaline in CRH KO mice compared to wild-type mice, although the levels of noradrenaline were slightly increased. Since CRH KO mice exhibit blunted glucocorticoid production, some fractions of the elevated plasma noradrenaline may arise from secretion by nerve terminals of the sympathetic nervous system. It is also possible that the adrenal medulla could contribute to elevation of plasma noradrenaline because the accumulation of adrenal noradrenaline might occur due to chronically impaired conversion of noradrenaline to adrenaline in CRH KO mice [12]. In the hearts of CRH KO mice, levels of the type 1 IP_3 receptor mRNA were also lower compared to their wild-type mates. After repeated immobilization stress for 7 days, levels of the type 1 IP_3 receptor mRNA decreased significantly [14], while levels of immobilized CRH KO mice remained the same as in CRH KO controls. This observation might suggest that glucocorticoid and/or adrenergic regulation is involved in this process.

In summary, we have found that the type 1 IP_3 receptors are downregulated when catecholamines are partially depleted. This effect can be realized through the α1AR and/or βAR. The present findings provide a molecular basis for the hypothesis that mechanism of the adrenergic modulation in the heart involves the type 1 IP_3 receptors.

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